Development of 2D & 3D culture models of neurogenesis using patient-derived stem cells to study neurodevelopmental diseases.

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Research Theme/Topic: Neurodevelopmental disease modelling using patient-derived stem cells

Background:

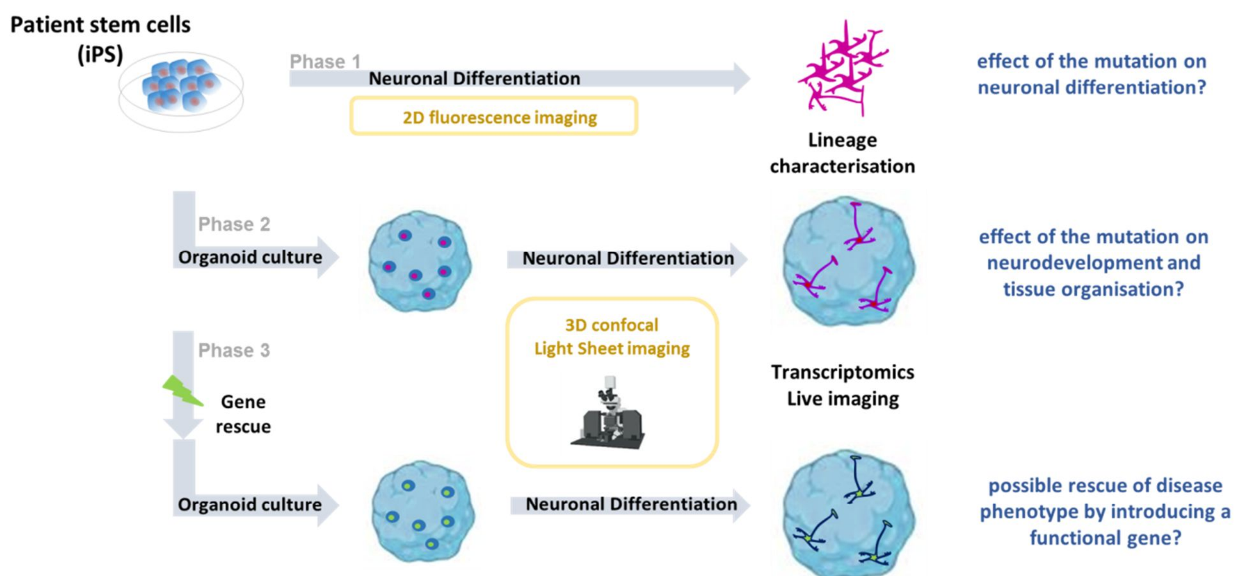
Despite recent progress in the techniques enabling the identification of genes responsible for neurodevelopmental pathologies, the mechanisms underlying the pathogenic phenotypes associated with these genes are still largely unknown. The use of induced pluripotent stem cells (iPS) derived from patients provides a substantial new tool to establish neuronal models in vitro, in order to analyse the role of specific mutations found in patients in the development of the disease. The capacity to grow these iPS cells in 3D models (organoids) gives a further capacity t recapitulate in vitro the early steps of brain development, and to identify which cellular processes are compromised by the mutations found in patients.

The aim of this project is to characterise neurons produced in culture from patients displaying a genetic neuropathology such as Joubert syndrome (JS), characterised by a cerebellar malformation causing significant neurodeficiencies. Using cellular reprogramming, pluripotent stem cells from patient will be analysed in vitro to discover the biological basis leading to brain malformation, in 3 experimental phases:

Phase 1: Starting from stem cells (iPS) reprogrammed from patient cells cultured in 2D monolayers, a neuronal differentiation protocol will be applied to evaluate the effect of distinct patient mutations on the differentiation efficiency. A protocol available in house will be applied in parallel to several cell lines from patients carrying different mutations to compare the associated neuropathological phenotypes, using established molecular and cellular assays.

Phase **2**: The neuronal differentiation protocol will be applied to patient iPS cells cultured in 3D, by producing organoids able to better replicate the process of neuronal cell maturation in vitro. The patient-derived organoids will be differentiated towards a cerebellar neuron phenotype, to analyse the cerebellar cell and tissue organization defects linked to each patient mutation. To analyse the differentiated cell types produced, immunodetection of set lineage markers will be combined with advanced 3D microscopy techniques including confocal and light-sheet technologies available in our dedicated imaging facility.

Phase **3**: The causal link between the mutation and the neuronal defects observed in patient cells will be tested by a rescue experiment, in which an intact copy of the mutated gene will be introduced into the patient cells, to assess whether the disease phenotype can be corrected. The wild type copy of the gene will be introduced by electroporation using molecular vectors designed for efficient gene expression in iPS cells, to determine whether the severity of the neuronal phenotype can be alleviated by introducing a functional copy of the gene.



Techniques involved include stem cell culture, cellular reprogramming technology, neuronal differentiation, 3D organoid production, disease modelling, neuronal marker detection, cell phenotyping, immunostaining, confocal & light-sheet fluorescence imaging with 3D reconstruction, transcriptome analysis, flow cytometry & cell sorting. Ongoing collaborations with 2 leading research groups in Paris and in Trento will provide opportunities for training visits and technology exchanges during the project.